Acta Oncologica Vol. 31, No. 6, pp. 684–697, 1992

Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Ischaemic treatment of liver cancer

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Liver cancer leads untreated rapidly to death. The ischaemic treatment of the liver has opened new palliative possibilities for patients with irresectable liver malignancies. The effect of ischaemic treatment was studied in the experimental model in the rat concerning the liver metabolic responses, the liver tumour growth, the liver tumour blood flow, the efficiency of a cytostatic agent and lipoidol, and the ultrastructural changes. In addition, the consequences of hepatic dearterialisation on glucose tolerance and insulin secretion were studied in patients with liver malignancies.

Hepatic dearterialisation induces hyperglycaemia, hyperinsulinaemia, and hypoglucagonaemia, but impaires the argenine-induced increase in insulin secretion. Further, the liver glycogen stores are decreased, probably due to an exaggerated glycogenolysis.

Hepatic dearterialisation reduces the hepatic activity of hepatic lipase probably due to an inhibition of de novo enzyme synthesis, reduces the oxidative capacity of the mitochondria, and inhibits the microsomal enzyme cytochrome (P_{450}) activity.

Hepatic artery or portal vein occlusion reduces the tumour growth rate, and causes similar decrease of blood flow in both normal and tumour tissue. In both tissues the decrease upon occlusion of the portal vein was more pronounced than upon occlusion of the hepatic artery.

The retarding effect of intermittent hepatic artery occlusion on tumour growth was not significantly potentiated with the additional treatment of MMC and lipoidol.

A gradual development of ultrastructural changes in the liver tissue started after 30 min of ischaemia. No difference was observed between hepatic arterial and portal venous ischaemia. Reflow adverses the morphological changes caused by ischaemia.

November 1991

Biokinetics and localization of some ^{111}indium-radiopharmaceuticals in rats at the macroscopic and microscopic level—An approach towards small scale dosimetry

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A detailed knowledge of the biokinetics and biodistribution of a radiopharmaceutical on the organ-, tissue-, cellular- and subcellular level is essential for a complete evaluation of the radiation dosimetry on the macro- and microscopic level. Such approaches towards small scale dosimetry, together with studies of possible biological effects, are of prime importance for the radiation dosimetry and risk estimates of many radionuclides, used in nuclear medicine, that emit conversion electrons and cascades of low-energy Auger electrons.

This thesis has focused on the long-term biokinetics and biodistribution of ^{111}In in rats, after administration of selected ^{111}In radiopharmaceuticals, for calculation of the mean absorbed doses to the human on the macroscopic level. The heterogeneous activity distribution in organs and tissues was investigated by quantitative, whole-body autoradiography, and the microscopic biodistribution was studied by light- and electron microscope autoradiography in the testes and bone marrow of the rat. The role of transferrin in the in vivo behaviour of ^{111}In is discussed.

The results both support and complement earlier findings of the behaviour and radiation dosimetry of ^{111}In, and present new and important information on the activity distribution, retention and absorbed doses on the macroscopic level. It is illustrated that quantitative, whole-body autoradiography is a valuable method for the evaluation of the biodistribution in various organs and tissues throughout the body, and in particular that ^{111}In accumulates in rapidly proliferating tissues. The activity distribution was found to be markedly heterogeneous in many tissues, such as the bone marrow, intestines, kidneys, liver, lymph nodes, spleen and testes. Light- and electron microscope autoradiography showed that indium accumulates in the interstitial tissues as well as in the seminiferous tubules in the testes, coincident with the radiosensitive spermatogenic cell lineages. Indium was found to be localized in both the cytoplasm and nuclei of cells in the testes and bone marrow.

December 1991

Radiation dosimetry using magnetic resonance imaging. Development of a dosimeter gel for measurements of 3D dose distributions in radiotherapy

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A new dosimetry system for 3D dose distribution measurements based on the Fricke dosimeter and magnetic resonance imaging (MRI) has been developed. The dosimeter consists of a ferrous sulphate solution incorporated in an agarose gel, which together constitute the dosimeter gel. The absorbed dose to the gel is measured by means of the proton spin-lattice relaxation rate, 1/T1 in an MR scanner. The dose distribution to an arbitrary slice within a dosimeter gel phantom can thus be determined.

The chemical yield of the dosimeter gel is significantly higher than that of the one for Fricke solution, and is strongly dependent on the initial ferrous sulphate concentration, assuming that the gel is bubbled with oxygen during preparation. A gel of 1.5 mM (Fe^{2+}) and 50 mM (H_2SO_4) has a sensitivity of $0.108\ s^{-1}Gy^{-1}$, corresponding to $G(Fe^{3+}) = 94\ (100\ eV)^{-1}$ and is linear up to 50 Gy. By decreasing (Fe^{2+}) G values of 170 $(100\ eV)^{-1}$ can be achieved, but the dose response is then only linear over a small interval (< 10 Gy). The dosimeter gel has uniform dose response over large volumes. Above 50 mM (H_2SO_4) the yield increases only slightly, but the gel strength decreases and results in gel phantoms with non-uniform dose response. Below 50 mM (H_2SO_4) the sensitivity of the dosimeter falls rapidly due to the decreased relaxivity of the ferric ions. The high chemical yield can be explained by a chain reaction and a reaction scheme is accordingly proposed. The dosimeter gel shows no dependence on dose rate or radiation quality and can be regarded as water-equivalent with respect to the interaction of the radiation. The diffusion coefficient of the ferric ions in the agarose gel is $1.91 \cdot 10^{-2}\ cm^2/h$. The diffusion blurs the dosimeteric image, but poses only a minor problem if the MR measurements are completed within the first two hours after irradiation.

Dose distribution data from external radiation therapy units have been determined using the dosimeter gel and MRI with good accuracy, but the precision is poor; about 5–10%.

December 1991

Developmental and clinical studies on germ cell tumors in childhood

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The present study was undertaken to examine some developmental and clinical aspects of pediatric germ cell tumors. The prognostic significance of immunohistochemical differentiation markers and serum AFP monitoring were evaluated in children with SCTs. In addition, to find out whether teratomas at various locations were associated with developmental abnormalities or with late sequelae of surgery, 77 patients operated on for an extragonadal or gonadal teratoma in childhood were studied after a mean interval of 20 years.

Immunohistochemically, the expression of cellular differentiation markers in benign SCTs closely resembled that observed in the corresponding normal mature tissues. However, some unexpected features were found in teratomas. Such features have recently been reported in normal fetal development. The similarities in cell differentiation between teratomas and fetal tissues may reflect the maturation taking place in these tumors. Assessment of the prognostic value of the differentiation markers disclosed that in no case did expression or lack of expression of the markers studied show any correlation with the clinical outcome. With regard to the classic histologic features of the tumors, the frequency of malignant recurrences was comparable in magnitude with that in previous reports; a malignant component in the primary tumor is a risk factor, but teratomas may undergo malignant transformation even when initially benign.

Reference values were established for serum AFP in infancy. In children with benign SCTs, at the time of diagnosis and during the 2-year follow-up period, serum AFP remained within the reference range. Malignant transformation of the teratoma caused elevation of the serum AFP concentration. The results of the present study are consistent with those of previous reports, which have shown that an increased concentration of serum AFP is a reliable marker of malignant transformation of teratomas. Moreover, after malignant transformation the proportion of the CNR subfraction of AFP decreased. Thus, in the monitoring of children with germ cell tumors, especially those with borderline values of serum AFP, ConA fractioning of AFP may give additional information.

The follow-up study of patients operated upon for a teratoma in childhood revealed various gonadal, vertebral, and/or urologic abnormalities, although the majority of the patients had no subjective complaints. Some of the late sequelae could be predicted from the anatomic location of the primary tumor. Where surgical access to the tumor is difficult, complications may occur and be difficult to rectify. Urologic late sequelae were observed in patients operated on for SCTs, especially in those in whom the primary tumor had an intrapelvic extension. Oral and mediastinal teratomas were also associated with abnormalities resulting from surgery.

Defects in segmentation or fusion of vertebrae seemed to be associated with extragonadal midline teratomas. Further, extrapelvic SCTs were often associated with subsequent development of spondylolysis in the lumbosacral spine. These vertebral abnormalities may be related to the effects of local tumor growth during development or, alternatively, may have resulted from the same developmental aberration that was responsible for the formation of the teratoma. In males, hypergonadotropic hypogonadism and sperm abnormalities were frequent and bore no relation to the location of the primary teratoma. The gonadal dysfunction found in males supports the hypothesis that the formation of a germ cell tumor and subsequent testicular dysfunction have a common etiology, possibly an underlying germ cell defect.

December 1991

Malignant germ cell tumours: Survival and side effects

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The prognosis of patients with malignant germ cell tumours treated at the Norwegian Radium Hospital is comparable to the results of other oncological centres:

- Non-seminomatous testicular cancer clinical stage I/IIA: Cancer-related survival is 100% (median observation time 70 months).
- Metastatic non-seminomatous testicular cancer: Crude 5-year survival is 90%.
- Testicular seminoma stage I: Cancer-related 10-year survival is 99%.

In 30 to 50% of the patients with very advanced disease, no curative treatment exists today. One major task is to improve the prognosis in this group.

The acute treatment-related toxicity is high for the majority of patients with malignant germ cell tumours, especially those receiving chemotherapy and/or radiotherapy.

With an observation time of three to seven years the most important long-term side effects are infertility, renal toxicity, Raynaud-like phenomena and neurotoxicity.

One of the major challenges in the treatment of malignant germ cell tumours today is to reduce acute and long-term toxicity. The present knowledge about risk factors for the development of particular side effects must, if possible, be taken into account in the treatment of individual patients.

Reduction of side effects must not compromise the principal aim of treatment: cure of an otherwise fatal malignancy.

Thorough and realistic information and counselling of patients and their families is important both before treatment and during the follow-up period.

Longer follow-up of patients will be necessary to assess all aspects of long-term side effects after treatment of malignant germ cell tumours. Present subclinical impairment may become of clinical importance in the patient's future life.

December 1991

Clinical and biomedical studies on adenocarcinoma of the uterine cervix

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Studies on adenocarcinoma of the uterine cervix have underlined the increasing incidence of this carcinoma, differences in its etiopathogenesis and natural history compared with cervical squamous cell carcinoma. The present study was undertaken to evaluate epidemiological, clinical and prognostic characteristics

(including DNA ploidy and S-phase fraction) of cervical adenocarcinoma, to study the levels of specific tumor markers, and to study the role of HPV 16 and 18 by using in situ hybridization and HPV peptide serology.

Epidemiological, clinical and prognostic factors were studied in 106 patients. The annual number of new cases and the mean annual age-adjusted incidence of cervical adenocarcinoma (mean 0.86 per 100 000) have been stable in Finland since the 1950s compared to the remarkable reduction of prevalence and incidence of cervical squamous cell carcinoma. The proportion of cervical adenocarcinoma of all cervical carcinomas was 20%. Twenty percent of the patients were nulliparous, 7.5% had diabetes, 20% had hypertension, and 56% were obese. Five patients (5%) had a history of previous subtotal hysterectomy. The main symptom was abnormal bleeding (78%). The diagnostic accuracy of cytologic examination and colposcopy were 61% and 64% respectively. The histologic pattern was pure adenocarcinoma in 87% and adenosquamous carcinoma in 13%. Fifty-five per cent of the tumors were well or moderately differentiated. In 57% the diameter of the tumor was less than 2 cm. The size of the tumor correlated with the grade of histopathologic differentiation and the presence of lymph node metastases. Lymph node metastases were found in 20% of the patients. Stage I was found in 61%, stage II in 22%, stage III in 16%, and stage IV in 1%. The overall 5-year survival was 65% (corrected 74%). Significant prognostic factors included the size of the tumor, nodal status, and stage. The 5-year survival rate was 85% for tumors of 2 cm or less, and 25% for tumors of 4 cm or over ($p < 0.001$), 79% for tumors with negative lymph nodes, and 32% for tumors with positive lymph nodes ($p < 0.001$), 79% for stage I tumors, 48% for stage II tumors, and 39% for stage III tumors ($p < 0.001$). In stabe Ib the patients treated with radiotherapy only had the worst prognosis. The histopathologic differentiation was not a significant prognostic factor in this analysis.

Aneuploid DNA pattern was found in 31% of the 125 patients studied, and most of the aneuploid tumors (51%) were triploid. DNA aneuploidy correlated with poor histopathologic differentiation, stage and tumor size. S-phase fraction was higher in aneuploid than in diploid tumors (mean 15% versus 4.5%). Below median (14%) S-phase fraction values were found more often in early stage tumors, both in the diploid and aneuploid groups. In Cox's regression model (multivariate analysis) both DNA ploidy ($p < 0.0002$) and S-phase fraction ($p < 0.007$) were independent prognostic factors together with tumor size ($p < 0.0001$) and nodal status ($p < 0.0001$).

Tumor marker determinations were performed in 42 patients. Pretreatment levels of CA 125 were elevated in 73%, CEA in 48%, serum TATI in 23%, and urine TATI in 38%. Elevated CA 125 levels were associated with histopathologic differentiation ($p = 0.002$). Elevated CEA levels were associated with nodal status ($p = 0.008$). CA 125 levels increased in 71% of the patients with progressive disease, CEA levels in 36%, serum TATI levels in 46%, and urine TATI levels in 20%. Regression of the disease correlated ($p < 0.05$) with stage, histopathologic grade, tumor size, and nodal status. In all patients with regressive disease the tumor marker levels decreased or stayed unchanged. Overall, CA 125 was the most sensitive tumor marker in the diagnosis and follow-up of the patients with cervical adenocarcinoma.

HPV 16 or 18 DNA was found in 18% of 106 patients studied by in situ hybridization. HPV 16 was present in 2 cases (2%), HPV 18 in 15 cases (14%), and both were present in 2 cases (2%). Of all HPV DNA positive cases, 88% were HPV 18 positive. HPV DNA positivity correlated with stage ($p < 0.01$) and tumor size ($p < 0.05$), but not with the grade of histopathologic differentiation, tumor proliferation defined as S-phase fraction, DNA ploidy, nodal status, or overall survival. These results suggest that HPV 18 is associated with cervical adenocarcinoma, but may not have a direct effect on survival.

Serum antipeptide antibodies (synthetic peptides No. 245 derived from HPV types 6, 11, 16 and 18 open reading frame E2) were measured by ELISA in 22 patients with cervical adenocarcinoma, in 22 patients with cervial squamous cell carcinoma, in 22 patients with ovarian cancer, and in 22 age-matched healthy controls. Compared to the controls, IgA and IgG antibody levels to the HPV 18 peptide were higher both in patients with cervical adenocarcinoma and squamous cell carcinoma. IgA antibody levels to the HPV 16 peptide appeared to be elevated mainly in patients with squamous cell carcinoma, while IgG antibody levels were elevated in both histologic type. No significant differences between cases and controls were found in IgA antibody levels to the HPV 6, HPV 11, or mumps peptides. IgA and IgG positivity for the HPV 18 peptide indicated an increased risk (relative risk 9.0) for cervical adenocarcinoma. In serial serum samples, IgA antibody levels to the HPV 18 and 16 peptides correlated with the clinical course of the disease both in patients with cervical adenocarcinoma and squamous cell carcinoma. These results show that patients with cervical adenocarcinoma develop a specific antibody response to the HPV 18.

In conclusion, there have been no changes in the incidence of cervical adenocarcinoma during recent decades in Finland. DNA ploidy and S-phase fraction are important prognostic factors together with the conventional prognostic factors and should be included to the routine diagnostic procedures. The determination of tumor marker levels may facilitate more aggressive treatment in cases with high or elevating tumor marker levels. HPV 18 is involved in the pathogenesis of cervical adenocarcinoma, as shown by in situ hybridization tests or by the determination of antipeptide antibodies.

December 1991

Genetic analyses of two inheritable tumour loci—Retinoblastoma and multiple endocrine neoplasia type 1

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Retinoblastoma and multiple endocrine neoplasia type 1 (MEN1) are two inherited cancer syndromes with autosomal dominant inheritance and high penetrance in affected families. Since these disease genes cause considerable morbidity within these families, it is of immense value to have genetic screening methods for early detection of predisposition for these tumours, so that appropriate steps can be taken.

Using polymorphic markers localized within the gene linkage analysis was performed on twenty retinoblastoma families, of which seven were Swedish. A LOD-score of more than 12 was established with no cross-overs, suggesting genetic homogeneity for this disease. The carrier status of individual members could be determined for all the families, and informed genetic counselling could be given based on this. We could detect one non-affected carrier in one family.

Patients with bilateral disease, which is known to be caused by constitutional mutations, were screened using Southern blot analysis with cDNA-probes for the retinoblastoma gene. Of 60 patients analyzed, we could detect and sublocalize 3 mutations affecting only parts of the gene. Unaffected siblings could be excluded as mutation carriers based on this analysis.

Twenty of the patients that exhibited normal results in the cDNA-screening were also analysed using pulsed-field gel electrophoresis. Of these twenty patients, one showed an abnormal restriction pattern. This was outlined and shown to be due to a constitutional balanced translocation (4; 13), with the breakpoint on chromosome 13 located within intron 17 of the retinoblastoma gene.

The gene for MEN1 has not yet been cloned. However, a locus has been defined on chromosome band 11q13. By using pulsed-field gel electrophoresis a detailed physical map has been constructed over this region and its physical size estimated. Comparisons between the physical distance and the genetic distance over the regions show a high frequency of meiotic recombinations at the MEN1-region.

January 1992

Studies on diagnostic evaluation of pancreatic carcinoma—Implications for surgery

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Biliary cytology detects over half of malignant biliary obstructions, and should be a routine extension of endoscopic cholangiography after demonstration of bile duct stricture. Pancreatic carcinoma is detected as accurately as biliary carcinoma. Bile aspiration may be attempted only if the more sensitive brushing fails technically.

Assay of CA 195 and CA 19-9 in pure pancreatic juice are of no diagnostic significance in pancreatic carinoma.

CT is highly recommended for staging of pancreatic carcinoma even in patients whose tumour has been detected in US, unless US already demonstrated unresectability. Contrary to this, US is not recommended in patients whose pancreatic tumour has been demonstrated by CT.

IFNAC differentiates between carcinoma and benign pancreatic diseases with 81% sensitivity and 96% specificity. Even so, in an individual patient the method may be seriously misleading. Thus, the justification of pancreas resection cannot always be based on cytology, but rather on the peroperative macroscopic appearance of the pancreas.

A minority of resectable pancreatic adenocarcinomas have an aneuploid DNA content as assessed by FCM, and about 10% of the cells are in the S-phase. Aneuploidy or high SPF are of no prognostic value in patients treated by pancreatic resection for pancreatic adenocarcinoma. DNA aneuploidy or high SPF, if detected in a resectable pancreatic tumour, should not indicate withdrawal from pancreatic resection.

January 1992

Progastrin in pancreas and the Zollinger–Ellison syndrome

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The Zollinger–Ellison syndrome consists of a severe gastric ulcer disease caused by one or more gastrin-producing tumours—gastrinomas. Most gastrinomas arise in pancreas which has always been somewhat puzzling as so far no gastrin production has been demonstrated in the normal adult pancreas. This is in contrast to other endocrine pancreatic tumours as for instance insulinoma, glucagonoma, and somatostatinoma which all have counterparts in the normal pancreas, i.e. cells that produce the hormone in question.

To investigate if the gastrin gene is activated in the normal pancreas, a new method for determination of gastrin was developed. In contrast to previously used radioimmunoassays, it registers all molecular forms of gastrin: both biologically active forms and inactive pre-stages independent of posttranslatoric processes. Several new antibodies were developed and used in combination with enzymatic treatment.

With the new method and other region-specific gastrin assays it was shown that the gastrin gene is activated in pancreas. Small amounts of progastrin is synthesized, but the posttranslatoric processing is inhibited and so only a small part is transformed to biologically active gastrin. During the fetal period, however, considerably more is transformed to active gastrin. In patients with pancreatic tumours—gastrinoma or adenocarcinoma—an increased progastrin-production was found in the normal pancreatic tissue.

By use of the new assay method it could also be shown that the processing of progastrin in gastrinoma tissue was considerably different from the normal processing in the gastric antrum. Often—and especially in patients with malignant pancreatic tumours—only a small part of the progastrin (10–20%) matured to biologically active gastrin. With the new assay also a large part of the inactive gastrin pre-stages could be demonstrated in the blood. Less than 50% of the circulating gastrin substances in patients with gastrinoma were biologically active gastrins. The new assay gives a more true picture of the gastrin production than conventional assays and will probably increase the diagnostic precision. Moreover, demonstration of large amounts of gastrin pre-stages in the blood may predict a malignant course of the disease.

January 1992

Epidemiologic studies of health hazards related to the Swedish art glass industry

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The production of glass began a few thousand years B.C. but was not introduced in Sweden until the 16th century. In general, throughout history, technical development has been rapid, but considering the art of glass blowing, methods used today are much the same as when the blowpipe was invented about 2 000 years ago. The composition of glass has changed, however, as a result of production specialization and the introduction of different types of glass (e.g. lead crystal, opal and heat-resistant glass).

Workers in the Swedish art glass industry are exposed to a variety of hazardous chemicals, some of which are known carcinogens. Most of these substances are components of the glass batch (i.e. the mixture of substances used to make glass), for example, compounds of arsenic, antimony, lead, cadmium, chromium, and nickel. Furthermore, large amounts of hydrofluoric acid and sulphuric acid are used in the polishing process, and, in the past, equipment made of asbestos was used for handling hot glass. Many of these chemicals are found not only within the glassworks themselves, but have also been emitted to the environment, causing general concerns about health hazards among people living in the vicinity of glassworks. Some glass workers are also exposed to combustion products and constant heat stress when working close to the glass furnaces.

The present studies show that Swedish art glass workers run increased risks of dying from cancer of the stomach, colon and lung and from cardiovascular diseases. In one of the studies, increased risks were also observed of prostate and pharynx cancer and of cerebrovascular diseases. The worker category showing the highest cancer risks was the glass blowers. The exposure route for this category of workers might be through both inhalation of airborne substances and ingestion of particles entering the mouth by way of the blowpipe. These possibilities are supported by hygienic measurements, including analysis of slag samples from inside the blowpipes.

The present studies were initiated due to concern about cancer risks expressed by people living close to glassworks, but the only increased risk found in this population was a cluster of brain cancers. Glass workers represent a large part of the population living in the vicinity of the glassworks, where the cluster of brain cancers occurred, and because of this, registry-based studies of Swedish glass workers seem to have spuriously indicated an occupational risk in this respect. Although the detected cluster of brain cancers might have its origin in some industrial discharge from the glassworks, the chemicals predominating in the emissions are not known to cause brain cancers, and other factors might be involved as well.

The cancer risks observed among glass workers can probably be explained by the known effects of several of the exposures occurring in glass production. The observed adverse effects on the cardio- and cerebrovascular systems may also be related to exposures at hand, first and foremost probably to lead exposure.

January 1992

Epidemiologic studies of nasal cancer and occupational exposures

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The object of the present research has been to elucidate the associations between malignant epithelial neoplasms of the nasal cavities and paranasal sinuses and workplace exposures to ascertained as well as suspected cancer causing agents and processes.

The research includes six independent case—control studies, located in various Italian regions. These regions are characterized by different patterns of industrialization, resulting in an involvement of various occupations of interest. Furthermore, the main findings of a national programme of surveillance of nasal cancer are presented.

The regions selected for the studies were the district of Biella in Piedmont (high proportion of the active population employed in textile industry), the province of Brescia in Lombardy (high proportion of the active population employed in metal industry), the provinces of Verona and Vicenza in Venetia (an area characterized by textile and leather industry and by farming), the province of Siena (a mainly rural area, with a well-established wood industry) and the province of Pisa (characterized by the presence of wood and leather industry); the two latter provinces are both located in Tuscany. The national surveillance program was based on the collaborative effort of 61 ear, nose and throat departments operating all over Italy. Finally, a case—control study involving subjects from the National Cancer Institute in Milan provided information at a national level, since patients from all over the country attend this centre.

The present research confirmed the well-established risk of nasal cancer associated with the occupations of the wood worker and leather worker. The combined estimates of the odds ratios were 6.0 (90% CI: 3.8–9.3) and 5.7 (90% CI: 2.8–12) respectively, which are lower and somewhat less dramatic effects than seen in some of the earlier studies.

A significant association between nasal cancer and some other occupations was detected, namely metal industry, the combined estimate of the odds ratio being 2.3 (90% CI: 1.2–4.3), textile and garment industry (OR: 2.2; 90% CI: 1.2–4.1), mining and construction industry (OR: 2.8; 90% CI: 1.6–5.0), farming (OR: 2.3; 90% CI: 1.4–3.8) and other occupations entailing exposures to dusts and fumes (OR; 2.0; 90% CI: 1.1–3.6).

The etiologic fraction associated with all hazardous occupational exposures was about 60%, indicating that a considerable preventive effect could be achievable by improving the quality of the work environment. With regard to the high fatality of this disease, and the serious impairment of quality of life among the survivors, the importance of prevention is not only obvious but also ethically imperative.

March 1992

The antibody response to papillomaviruses

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The local and systemic antibody responses against papillomavirus were studied in women with papillomavirus-associated diseases. Papillomavirus epitopes were identified and characterized for cross-reactivity between different papillomaviruses. A local immune response to papillomavirus (PV) early and late antigens in cervical secretions from women with PV-associated disease was found to exist. The cervical immune response had a better correlation to cervical disease than the serum antibody response to the same PV antigens. Women with CIN were found to have elevated IgA antibody titers to BPV compared to patients with condylomas or healthy controls. Women with CIN or cervical carcinoma were shown also to have slightly elevated serum IgA antibody titers to BPV in serum. Using synthetic peptides, women with CIN or carcinoma were found to have elevated serum IgA antibodies to a synthetic peptide derived from the E2 ORF of HPV 16, whereas women with condylomas only had elevated antibody responses to this peptide in their cervical secretions. The putative E2 protein was detected in HPV 16-carrying cell lines and biopsies using both antipeptide polyclonal and monoclonal antibodies and peptide-purified antibodies from human sera. The antigenic epitopes of the HPV 16 capsid proteins L1 and L2 were mapped using 66 overlapping 20 residues synthetic peptides in ELISA for detection of IgA, IgG and IgM antibodies in sera from women with HPV 16-carrying cervical carcinoma. Regularly IgA and IgG reactive epitopes were identified in an internal region of L2, in the carboxy-terminus of L1 and in an internal highly conserved region in the L1 protein. Broadly cross-reactive PV epitopes shared by PVs from cow, dog, chaffinch and man were identified within the capsid proteins by testing HPV 16-derived synthetic peptides for reactivity with hyperimmune sera and monoclonal antibodies against purified animal PVs. The cross-reactive epitopes were located at the carboxyterminus and in an internal region of L1. The most reactive epitope had the sequence DTYRF and was located at the carboxyterminus of L1. Antisera against HPV 16-derived peptides containing the 3 major broadly cross-reactive epitopes reacted with bovine PV in ELISA and one of these

antisera was broadly reactive with the capsid antigen of different PV types in immunohistocytochemical staining.

April 1992

Chemotherapy in rats with an implanted liver carcinoma—Studies on manipulation of dietary protein and of blood flow in combination with 5-fluorouracil or doxorubicin.

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Rats with an implanted carcinoma in the liver were treated with 5-fluorouracil or doxorubicin. The effects of manipulation of dietary protein or of blood flow were studied in order to increase the antitumor effect and/or to reduce the adverse effects on normal tissues. The incorporation of labelled 5-fluorouracil or orotic acid into target molecules was analysed as well as liver nucleotide profile and tumor growth.

Following protein deprivation, the incorporation of a therapeutic dose of 5-fluorouracil into liver and intestinal RNA increased. This was prevented by giving essential amino acids parenterally. Protein deprivation did not significantly affect 5-fluorouracil incorporation into tumor RNA.

In non-tumor bearing rats, protein deprivation also increased the incorporation of the normal precursor orotic acid into liver and renal RNA. The increase was prevented by giving essential amino acids, either parenterally or orally. The decrease in liver nucleotides and body weight was similarly reversed.

Occlusion of the portal vein for 30 min in rats without a tumor caused a considerable decrease in liver and intestinal nucleotides but an almost complete restitution was seen within 6 h of reperfusion.

Occlusion of the portal vein or of its branch supplying the tumor-bearing lobe was combined with 5-FU infusion into the hepatic artery. The results indicated a decreased anabolism of 5-FU in the tumor and an increase in systemic exposure.

The combination doxorubicin + hepatic artery ligation gave a greater antitumor effect than either of the treatments alone.

Addition of norepinephrine decreased body weight loss and possibly gastrointestinal toxicity caused by simultaneous administration of doxorubicin and degradable starch microspheres via the hepatic artery.

April 1992

Scintigraphy of the reticuloendothelial system—Studies of visceral blood flow and of red bone marrow activity and distribution

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The reticuloendothelial system (RES), is an important bodily defence system mainly located in the liver, spleen and bone marrow. The reticuloendothelial (RE) cells are macrophages located in close contact with the sinusoids in these organs. The RE cells have common characteristics and are identifiable by their ability to ingest particulate matter by means of phagocytosis. This thesis deals with RES scintigraphy, which is a method for assessing morphology and function of the RES in the liver, spleen and bone marrow after intravenous injection of radiocolloids.

In this thesis attempts have been made to assess information from components of the reticuloendothelial system that are not usually included in an RES scintigraphic examination. The interest has been focused on visceral blood flow, pelvic bone marrow activity, red bone marrow extension, and focal bone marrow reconversion.

RES scintigraphy including a visceral radionuclide angiography (RNA) was performed in 1 230 patients. A generalized increase of arterial blood flow to the liver was found in 70 patients. In 27% of the 70 patients the static liver-spleen recordings were normal or possibly pathologic. Alcoholic liver disease and liver metastases were the commonest cause of liver arterialization.

Twenty-one consecutive patients with alcoholic liver disease were also studied with RES scintigraphy and RNA. Fourteen had increased arterial blood flow to the liver.

The occurrence and degree of bone marrow extension (BME) and pelvic bone marrow activity values (BMA) were investigated in patients with prostatic carcinoma, alcoholic liver disease, polycythemia vera (PCV) and secondary or relative polycythemia (PS). BME and increased BMA were found in almost all patients with alcoholic liver disease and PCV. In general, there were marked BME and high BMA values. Among the prostatic carcinoma patients, with or without bone metastases, 79% had BME, but to a lesser degree compared with alcoholic liver disease and PCV. Patients with widespread metastatic disease had the highest grade of BME. Eight prostatic carcinoma patients had no extension. All PS patients had normal BMA values, but 11 had BME.

In 20 lesions of Paget's disease of bone, normal or increased radiocolloid uptake was found in 15 and decreased uptake was found in 4.

Increased radiocolloid uptake was found in one patient with an osteonecrotic knee lesion.

In conclusion, important diagnostic information is obtained if RNA, BMA and BME are included in the RES scintigraphic examination. —RNA increases the detection rate of liver pathology. —Increased BMA and BME are prominent features in alcoholic liver disease and PCV. —BMA and BME are valuable in differentiating PCV from other polycythemias. —BME is common in prostatic carcinoma patients and increases the risk of peripheral metastases. —A preserved RE function in a pagetic lesion contradicts malignant disease as a differential diagnosis. —A spontaneous osteonecrosis of the knee promoted a focal reconversion to marrow with phagocytic capacity.

May 1992

Carcinogenesis studies in human normal and transformed buccal epithelial cells

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Oral cancer is frequent in South-East Asia, where chewing of betel quid is a common habit. Whether areca nut, one of the main ingredients in the quid, is carcinogenic is not clear. The use of defined culture methods allows experimental studies directly in human cells from the target tissue. Therefore, this study was undertaken to develop serum-free culture conditions for normal human buccal epithelial cells, characterize growth regulation and longevity of these cells, attempt to transform them, perform comparative studies with tumor-derived cells, and investigate effects by areca nut related to early events in multistage carcinogenesis.

For primary cultures of normal buccal epithelial cells, a growth factor supplemented medium, EMA, based on MCDB 153, in combination with fibronectin/collagen matrix was optimal. At

these conditions, secondary cultures could be maintained up to 10 passages (about 200 days) and expanded up to 86×10^3 times. The cells also had normal, basal cell features, such as diploid karyotype, keratin pattern, abundant microvilli, few desmosomes and tonofilament bundles. Several defined factors stimulated growth of the cells, which generally correlated with decreased cell surface area. The ability to undergo squamous terminal differentiation was retained by the cells. Serum was potent in accelerating differentiation as judged by decreased growth and migration, in addition to increased cell area, involucrin and cross-linked envelope formation. Ca^{2+} at 1 mM also induced differentiation. Transforming growth factor $\beta 1$ (TGF-β) was primarily growth inhibitory without significant effects on differentiation. The SqCC/Y1 buccal carcinoma line was successfully maintained in EMA over a year. This apparently immortal line was found to be resistant to differentiation by serum, Ca^{2+} and TGF-β, aneuploid and tumorigenic in animals.

An aqueous areca nut extract was found to be cytotoxic, induce differentiation in the normal cells, and cause loss of microvilli with formation of plasma membrane ridges, and indications of internalized extract particles. The extract was also genotoxic and the DNA lesions accumulated with time, possibly as a result of continuous exposure to residual particles and/or inhibition of DNA repair. The extract caused similar cytotoxicity in the differentiation-resistant SqCC/Y1 line as the normal cells, indicating that toxicity and induced differentiation by the extract could occur independently. Of several areca nut-specific compounds investigated in normal cells, 3-(N-nitrosomethylamino)-propionaldehyde (NMPA) was the most potent on a molar basis, and significantly decreased growth, content of low-molecular-weight thiols, and caused DNA damage, at 0.3 mM. About 10-fold higher concentrations of arecoline, guvacoline or N-nitrosoguvacoline was required to cause similar toxicity. Structure-activity comparisons indicated that the aldehyde moiety and/or reactive metabolites of NMPA may be responsible for its toxic potency.

Transfection of normal buccal epithelial cells with the SV40 large T antigen (SV40T) gene yielded several hyperdiploid strains with extended life spans, and one pseudodiploid, benign strain which is apparently immortal. The immortal line was markedly more resistant than normal cells and strains with extended life spans, to the growth inhibition and differentiation by serum and TGF-β. All strains expressed SV40T, indicating possible Rb and/or p53 inactivation, that could confer the extended longevity observed.

Normal cells with ability to undergo differentiation, experimentally transformed cells with premalignant phenotypes, and tumor-derived malignant cells from human buccal epithelium can now be studied at the similar serum-free conditions. These cells are valuable tools for future research on the multistep nature of carcinogenesis. This thesis also showed cytopathogenic effects of areca nut agents, notably NMPA, in the concentration ranges found in saliva, strongly indicating a contribution of areca nut in betel quid carcinogenesis.

May 1992

On the prognosis and treatment of early stage endometrial carcinoma—Studies with special reference to uterine papillary serous carcinoma

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The prognosis and therapy in the subgroup of endometrial malignancies called uterine papillary serous carcinoma (UPSC) was investigated. This entity constitutes about 6–8% of all endometrial carcinomas clinical stage I but, as has been shown here, accounts for one-third of the cancer mortality in the early stages of endometrial carcinoma.

- The prognostic significance of nuclear atypia, FIGO grade and age was determined in patients with clinical stage I–II uterine papillary serous carcinoma.
- DNA-index and S-phase fraction, determined by flow cytometry on formalin-fixed, paraffin-embedded endometrial curettage material was evaluated in relation to nuclear atypia, FIGO grade and age in early stage endometrial carcinoma.
- A new method of minimizing the dilution of non-epithelial cells in the malignant curettage material when performing flow cytometry was developed.
- The survival and pattern of metastasis in a population-based patient material was investigated.
- The effects on survival of a new aggressive treatment regimen was assessed.

The results show that: Patients with uterine papillary serous carcinoma have a much worse prognosis than do patients with ordinary endometrial carcinoma. DNA ploidy and S-phase rate derived from flow cytometry performed on disintegrated, previously paraffin-embedded, endometrial curettings are important prognostic factors.

It is possible to exclude contaminating non-epithelial cells from the DNA analysis by the use of an anti-cytokeratin antibody.

Patients with uterine papillary serous carcinoma clinical stage I have a significantly higher risk of recurrence than patients with non-UPSC. The localisation of the recurrences among the UPSC patients is more similar to that of serous papillary ovarian carcinoma than that of ordinary adenocarcinoma of the endometrium. A primary treatment of UPSC with a more extensive surgery, adjuvant external radiation and platinum-based chemotherapy seems to improve the outcome.

May 1992

Cancer of the uterus—The value of MR imaging in primary and recurrent disease and its potential impact on patient management

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Analysis of MR examinations in 365 patients formed the database for this thesis. Surgical–pathologic correlation was available in 108 patients with endometrial cancer, in 46 patients with benign uterine disease, and in 88 patients with carcinoma of the cervix. In 66 patients with carcinoma of the cervix the initial diagnosis was confirmed by histology and MR findings were correlated with patients outcome at six and twelve months following radiation therapy. An additional 57 patients were irradiated for carcinoma of the cervix and in these patients MR findings of residual tumor or radiation changes were compared with clinical evaluation, cytology, and/or histology. MR interpretation was performed independently of surgical–pathologic or clinical findings and included analysis of unenhanced as well as contrast-enhanced images.

In patients with endometrial carcinoma, sensitivity of MR imaging for detection of an endometrial abnormality was 74% for unenhanced scans and 88% for contrast-enhanced scans ($p < 0.005$). Although submucosal leiomyoma could be differentiated from other endometrial abnormalities on MR scans, endometrial polyps could not be consistently differentiated from

endometrial carcinoma, and histology remains essential. MR accuracy for staging of endometrial cancer was 65% for unenhanced T1- and T2-weighted images, and it improved significantly to 82% with contrast-enhanced scans ($p = 0.024$). Contrast enhancement also significantly improved the evaluation of the depth of myometrial invasion. Analysis of unenhanced scans yielded a positive predictive value for deep myometrial invasion of 75%, whereas analysis of contrast-enhanced scans yielded 95% ($p = 0.015$).

In carcinoma of the cervix, tumor detection and estimation of tumor size and depth of stromal invasion was excellent on unenhanced T2-weighted images. Although tumor size was consistently overestimated (by 13%) as compared with size measured at pathology, the overestimation was consistent (correlation coefficient $r = 0.977$). In the assessment of depth of stromal invasion the overall accuracy was 78%, significantly better than the 38% obtained from unenhanced and contrast-enhanced T1-weighted images only. However, MR was not useful in the evaluation of tumors confined to endocervical glandular tissue. An MR imaging assessment of deep stromal invasion was correct with a probability of 0.85. In staging carcinoma of the cervix, the highest accuracy (84%) was achieved when unenhanced T1- and T2-weighted images were used. There was a significant decrease in accuracy (to 72%) when unenhanced and contrast-enhanced images were analyzed. The use of contrast media helped only in advanced cases, mainly in the evaluation of adjacent tissue invasion.

In MR evaluation of malignant lymphadenopathy, the small number of malignant nodes (present in only 31 patients) may have falsely inflated the high accuracy rate of 91%. The sensitivity for detection of malignant nodes was disappointingly low at 68%. Neither unenhanced nor contrast-enhanced images could differentiate enlarged benign from malignant nodes.

MR imaging was shown to be a powerful prognostic tool in patients with cervical carcinoma undergoing radiation therapy. The most important prognostic factor in univariate and multivariate analysis was the presence of lymph node metastasis, but MR findings of tumor stage, tumor size, and invasion of the parametria also correlated with patient prognosis. The use of MR in concert with clinical evaluation allowed pretreatment identification of low- as well as high-risk patients, contributing to better prognostic assessment and a more rational therapeutic approach, particularly with respect to both radiation treatment planning and the use of adjunctive systemic chemotherapy.

Finally, in the irradiated pelvis, the overall accuracy of MR in differentiating between radiation changes and residual tumor was 78%. The positive predictive value in patients studied during the first six months after initiation of radiation therapy was only 57%, indicating that physical examination and biopsy remain essential in patient evaluation.

May 1992

Malignant mesothelioma—The prognostic significance of biological and clinical characteristics

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From 1960 to 1990, 163 patients with confirmed malignant mesothelioma were registered at the Helsinki University Central Hospital. Studies were performed retrospectively on those registered up to 1980, and prospectively on those registered between 1981 and 1990. The ratio of male to female patients was 2:1 between 1960 and 1980, and almost 4:1 between 1981 and 1990.

Forty-six per cent of patients registered between 1960 and 1980, and 41% of patients registered from 1981 to 1990 were either not known or not likely to have been exposed to asbestos. After mineral fibre analysis, the latter finding was reduced to 30%. The main fibre type was crocidolite/amosite, found in 15/27 lung tissue specimens. It was also found in 4 patients with malignant mesothelioma who had been exposed mainly to anthophyllite fibres, indicating that there may be a carcinogenic role for anthophyllite. In both periods, the main symptoms at presentation were dyspnoea, cough and chest pain; the median delay in diagnosis was 3 months. For the retrospective study, good performance status (WHO 0-1) and early clinical stage were found to be independent favourable clinical prognostic factors. For the prospective study, diagnostic delay of more than 6 months, epithelial histology and female gender were also found to have favourable prognostic significance.

Five locally-aggressive hemithorax multi-modality treatment programmes were prospectively evaluated, using high-dose hemithorax irradiation schedules combined with chemotherapy, after debulking surgery. A new system for evaluating tumour responses in pleural mesothelioma was used. None of the combined treatment programmes prevented local invasive growth, or the spread of mesothelioma outside the hemithorax. The median survival time remained unchanged between 1981 and 1990, despite changing treatment policies. However, survival was increased, from 8 to 12 months, for those patients who completed the protocol treatment. This indicates the tumour's resistance to both chemotherapy and radiotherapy, although there is some effect of treatment. The results obtained from the 5 programmes only indicate trends: they cannot be properly compared using statistical methods.

The favourable experimental prognostic factors were: low total fibre concentration ($< 1 \times 10^6$ f/g dried lung tissue), anthophyllite as the main fibre type; and normal chromosome 7. In addition to identifying these experimental prognostic factors in univariate analyses, the results suggested that the size of the S-phase fraction (as assessed by DNA flow cytometry) had independent prognostic significance.

There was a correlation between high total fibre concentration, and partial or total loss of chromosomes 1, 4, or 9, or chromosomal rearrangements involving a breakpoint at 1p11-p22. There was also a correlation between crocidolite/amosite as the main fibre type, and partial or total loss of chromosomes 1, 3, or 4 or chromosomal rearrangements involving del(3p). Diploid cells predominated in 39 out of 63 tumours analysed. Despite the recent rapid increase in the number of cases, mesothelioma remains a rare disease. For this reason it is essential that carefully-designed multi-institutional phase III trials are carried out. However, no phase III trials can be contemplated until an effective treatment is found. Our phase II studies contribute to the understanding of effective treatment design for mesothelioma. The identification of prognostic factors, both clinical and experimental, is important for stratification in future therapeutic trials, and for directing treatment strategies. However, more data are needed to confirm the significance of the experimental factors. In conclusion, this study assessed the relative importance of various prognostic factors for the course of malignant mesothelioma. The results offer a basis for stratification in the design of treatment trials for this disease.

May 1992

Aspects of platelet-derived growth factor and its receptors in normal growth and oncogenesis

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The present investigation focuses on certain aspects of platelet-derived growth factor (PDGF) and is based on results obtained from in vitro and in vivo studies.

The first part of this investigation concerns negative regulation of cell growth. This study shows that the synthetic double-stranded RNA poly I:C, is an effective inhibitor of PDGF- and epidermal growth factor (EGF)-stimulation of quiescent fibroblasts in culture. The effect of poly I:C on proliferation was found to be, at least in part, exerted by a soluble factor possibly IFN-β or an IFN-β related molecule.

Next, aspects of PDGF and PDGF receptor expression in tumor cell lines is discussed. A non-small lung cancer cell line (H-157), previously shown to express PDGF mRNA, was found to possess functional PDGF-β-receptors. This suggests that an autocrine pathway may operate in these cells. However, the ability of exogenously added PDGF-BB to induce receptor autophosphorylation suggests that the system is not saturated.

The production of PDGF from tumor cell lines might reflect properties of the normal counterparts of the tumor cells. As a model to study the expression of PDGF from cells of the basophil/mast cell lineage, the cell line KU 812 was used. KU 812 cells were found to constitutively express PDGF, an expression that was enhanced when the cells were induced to differentiate.

An objective of this study was to investigate if PDGF can elicit a stroma reaction when released by tumor cells. A human melanoma cell line (WM 9), stably transfected with an expression vector for PDGF-B, was injected subcutaneously to *nu/nu* mice and found to yield tumors with a connective tissue framework, an abundance of blood vessels and no necrotic areas. Since mock-transfected WM 9 cells gave rise to necrotizing tumors without stroma, it is suggested that PDGF is a potent inducer of tumor stroma.

As a model to study the in vivo effects of PDGF, transgenic mice carrying a PDGF-B construct under control of the promoter for myelin basic protein (MBP) were generated. RNA analysis of the transgenic mice revealed expression in brain tissue. In several of the transgenic lines, phenotypic abnormalities were observed, microphtalmia and macrophthalmia being the most common. Earlier studies have shown that PDGF is present in the eye and the MBP/PDGF-B transgenic mice might provide a model for study the involvement of PDGF in eye development.

May 1992

c-src expression in neuroendocrine tumors–Neuronal splice variants of c-src as diagnostic and prognostic markers in neuroblastoma

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The protein tyrosine kinase $pp60^{c\text{-}src}$ and its neuronal form $pp60^{c\text{-}srcN}$ have been implicated to be involved in neuronal differentiation, and the maintenance of the mature neuronal phenotype. In this study a correlation between neuronal differentiation and c-src kinase activity was found in human neuroblastoma and SCLC cell lines. Furthermore, $pp60^{c\text{-}srcN}$ was only detected in neuroblastoma cells and not in SCLC cells or non-neuroendocrine tumor cells.

An increase in specific c-src kinase activity was found in cultured human neuroblastoma cells induced to differentiate neuronally. The activation was an early event, seen already 6 h after induction. The ratio between $pp60^{c\text{-}src}$ and $pp60^{c\text{-}srcN}$ remained unaltered in differentiated cells. Furthermore, the increase in specific c-src kinase activity was not a result of a change in tyrosine phosphorylation of the c-src proteins.

The expression of $pp60^{c\text{-}src}$ and $pp60^{c\text{-}srcN}$ was also studied in childhood tumors. $pp60^{c\text{-}srcN}$ was only detected in neuroblastomas, ganglioneuroblastomas, and retinoblastomas. Infant neuroblastomas with favourable prognosis expressed high levels of $pp60^{c\text{-}srcN}$ in spite of their morphologically primitive phenotypes. We concluded that $pp60^{c\text{-}srcN}$ could be of diagnostic and prognostic value in neuroblastoma.

The expression of the neuronal markers neuron-specific enolase and synaptophysin correlated to high expression of $pp60^{c\text{-}srcN}$. These findings supported the notion that morphologically immature neuroblastoma cells in infant tumors showed biochemical signs of neuronal differentiation.

Three c-src mRNA species (c-src, c-srcNI, and c-srcNI + NII) have previously been found in human brain. All three mRNA forms could be detected in neuroblastomas, while phenochromocytomas expressed only the c-src and c-srcNI + NII transcripts. Analysis of the chromaffin cell-specific enzyme PNMT revealed that all neuroblastomas were negative for this enzyme, while pheochromocytomas and adrenal medulla were positive. Thus, there was no evidence for a chromaffin differentiation in the neuroblastoma cells.

May 1992

Human intestinal alkaline phosphatase–Tissue expression and serum levels.

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Human alkaline phosphatase (ALP) comprises four isozymes, viz liver/bone/kidney or tissue unspecific (AP), intestinal (IAP), placental (PLAP) and germ cell or PLAP-like alkaline phosphatase, with their main expression in specific tissues as indicated by their names. The isozymes are coded by different genes, but they are closely related, with more than 50% amino acid sequence homologies. Their biological function is unclear. In certain malignant and benign diseases, serum elevations of one or more of the isozymes occur, which is of diagnostic importance. In this study, the special expression of the intestinal isozyme in human tissues and sera, in normal as well as in pathological conditions, has been investigated by use of isozyme specific monoclonal antibodies.

Monoclonal antibodies against the AP, IAP and PLAP isozymes were prepared, and specific assays developed, based on these monoclonal antibodies and the catalytic activity of the isozymes. By use of these assays the basal levels of all three isozymes were examined in selected normal organs. The isozymes were found to be expressed in measurable amounts in all the examined organs.

IAP was immunohistochemically localized to the epithelial cells of membranes lining the ducts and tubules of the kidney, liver, pancreas and small intestine.

Normal human serum contained all three isozymes. The AP isozyme constituted about 90% of the total ALP activity, the IAP isozyme less than 10% and the PLAP isozyme about 1%. Considerable interindividual variations of the serum IAP activity were observed. The serum activities of the IAP isozyme were related to

the individual AB0 blood group and secretor status. Non-secretors had low levels of IAP activity amounting to about one-tenth of the activity in sera from blood group B or 0 secretors, while blood group A secretors had serum IAP activities in the same order as non-secretors. High individual day to day variations were observed.

Fat absorption caused serum IAP to increase significantly for all persons, but it was rapidly cleared from the blood. We found that the release of IAP into the blood was linked to lipid absorption, but removal from the blood was not linked to lipoprotein clearance.

Certain tumors of the testis expressed elevated levels of all three ALP isozymes. The highest activity of IAP was observed in one yolk sac tumor, in agreement with the endodermal origin of this tumor. In seminoma tissue the AP and PLAP isozymes were significantly, and IAP moderately, elevated.

Cirrhosis of the liver caused significantly increased serum levels of IAP besides the AP isozyme. In inflammatory diseases of the small intestine, normal serum IAP activities were observed.

May 1992

Optimization of radiation therapy planning using physical and biological objective functions

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The aim of radiation therapy is to eradicate all clonogenic tumour cells without causing severe injury in normal tissues. Optimization of treatment planning means finding the most preferable treatment prescription. During the recent years new methods for optimization that aim at solving the inverse problem in radiation therapy have been developed. In contrast to classical optimization, where only a limited number of degrees of freedom are considered, such as beam weights and angles of incidence, the recently developed techniques work with a large number of degrees of freedom. In radiation therapy, the objective function subject to optimization can be either physical or biological. In this work optimization is performed using both physical and biological objective functions. Biological models that describe the response of tumours and normal tissues to heterogeneous dose delivery have been developed. Optimization is performed by maximizing the probability of achieving complication-free tumour control. The accuracy and applicability of biological optimization are dependent on accurate radiation response data. Clinical data is already available for many organs and tumours. Biological optimization is a promising line of development, and will probably dominate treatment planning in the future.

May 1992

Radiation-induced pneumonitis—Clinical and experimental studies with special emphasis on the effect of smoking

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Bronchoalveolar lavage (BAL) is an established method providing diagnostic support and evaluation of disease activity in interstitial lung disease (ILD). The aims of the present investigation were 1) to study the inflammatory response in pneumonitis evoked by irradiation. 2) to evaluate how well lung tissue inflammation is reflected in BAL findings. 3) to study the effect of smoking on radiation-induced pneumonitis.

BAL was performed in 21 patients (11 smokers, 10 non-smokers) who were treated for breast cancer, stage 1 (T1M0N0) by postsurgery irradiation to an accumulated target dose of 56 Gy. It was found that irradiation induced an alveolitis in the non-smoking patient group while the smoking patients did not differ from their smoking controls. The alveolitis in non-smokers was characterized by an increase in lymphocytes, mast cells and elevated concentrations of hyaluronan (HA), and fibronectin (FN). Three of the non-smoking patients had chest x-ray infiltrates indicating the presence of pneumonitis.

An animal experimental model for radiation-induced pneumonitis and fibrosis was established in rats, allowing comparative analysis of BAL fluid and morphology. In the rat model a divergence was noted between the differential cell counts in BAL and cells observed in the interstitial tissue, which was most notable for neutrophils (PMN) and mast cells whereas there was a good correlation between HA content in BAL and HA deposition in the lung tissue. A marked infiltration of intraseptally-located mast cells occurred during the pneumonitis-phase, and this increase was paralleled by a deposition of HA in the interstitial tissue. Histochemical fixation and staining properties of the mast cells revealed that the majority of these cells were of connective tissue mast cell type (CTMC). Compound 48/80, a mast cell secretagogue, significantly altered the HA content both in BAL and in lung tissue in the irradiated animals. Regular treatment throughout the whole experimental period induced depletion of mast cell granules and a decrease in HA deposition whereas 48/80 treatment during the pneumonitis phase enhanced HA deposition.

A rat model with smoke exposure was developed, and the effect of cigarette smoke on radiation-induced inflammation was studied. Rats that smoked 3 weeks prior to irradiation and continued to smoke throughout the observation period (7 weeks) had a significantly reduced inflammatory response compared to irradiated non-smoking rats. The most prominent BAL findings in the smoke-exposed rats were a decrease in PMN, mast cells and a decrease in HA.

In conclusion, irradiation induces an alveolitis characterized mainly by mononuclear cells. Mast cells seem to be of importance in the remodelling of the connective tissue in the radiation-induced inflammatory response. Hyaluronan is an important component in the early connective tissue response preceding later collagen deposition, and its interstitial deposition is very well reflected in BAL. Moreover, tobacco-smoke suppresses the radiation-induced inflammation with a decreased recruitment of effector cells including mast cells.

May 1992

Endocrine treatment of prostatic adenocarcinoma—An experimental study in the rat

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The present study was designed to evaluate and compare effects of different endocrine treatments in two rat prostatic tumor models (Dunning R3327 and AT-1). The aim was to investigate if treatments with estrogens or gestagens affect tumor growth and morphology, by mechanisms not related to the suppression of androgens.

Treatment of castrated rats bearing prostatic adenocarcinomas with 50 μg estradiol-17β (E2) s.c. daily inhibited tumor growth more effectively than castration alone, and the number of epithelial cells was reduced. Castration alone decreased the tumor epithelial cell size, while the number of cells was the same as in tumors from intact animals. Anti-estrogen, tamoxifen, did not block the inhibitory effect of E2 on tumor growth rate in castrated rats, suggesting that the effect of E2 is not mediated by interaction with estrogen receptors on the tumor. In a dose–response study on the effects of E2 in castrated and testosterone-supplemented rats, a dose of 10 μg s.c. daily or higher was needed to inhibit growth. In intact (non-castrated) animals, a dose of 1 μg E2 s.c. daily was sufficient to inhibit the tumor growth rate. These findings indicate that the suggested direct effect of estrogen on prostatic tumors is present at a 10-fold higher dose level than the anti-gonadotrophic effect of estrogen.

In order to study the effect of E2 on the tumor growth rate in the hormone-insensitive state, two tumor models were used: the Dunning R3327 relapsed after castration and the androgen-independent, anaplastic Dunning AT-1. In both models E2 treatment suppressed the tumor growth rate and decreased the tumor mitotic index. In the relapsed Dunning R3327 model, E2 partly prevented the dedifferentiation and increased the number of apoptotic cells, suggesting an increased cell death rate. The present work also demonstrated that estrogens had stimulatory effects on the stromal compartment of prostatic tumors. In an attempt to clarify the stimulatory effects of E2, the occurrence of different cytoskeletal and extracellular matrix proteins was studied using immunohistochemistry. The amounts of collagens I and III and fibronectin were considerably elevated in the tumor stroma after E2 treatment. Cytokeratin 14, a marker for basal epithelial cells, was reduced by E2 treatment of castrated rats.

Two gestagens, medroxyprogesterone acetate (MPA) and 6-methylene-4-pregnene-3,20-dione (6MP), were used to treat experimental prostatic adenocarcinoma, and both substances had growth inhibitory effects on the tumor. Combined treatment with E2 and MPA caused more inhibition than E2 treatment alone. These results support the idea that gestagens have direct inhibitory effects on prostatic adenocarcinoma. It was also shown that the decrease in tumor blood flow after castration was conteracted by treatment of the animals with 6MP.

The results in the present thesis show that estrogens and progestagens have tumor-inhibitory effects on prostatic adenocarcinoma which are not related to suppression of androgens.

June 1992

Studies on the mechanism for interferon induced tumor cell resistance to natural killer cell activity—Can MHC-associated molecules be a clue?

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The mechanisms for how NK cells recognize their target cells and how interferon treatment of the target can modulate this event is not clear.

This study shows that interferon (IFN) can inhibit the ability of target cells to activate or trigger the cytolytic machinery of the NK cells.

IFN-treatment is also shown to reduce target sensitivity to IL-2 activated lymphocytes, LAK cells, with no distinction made between LAK cells of NK or T cell type. Thus, NK cells and LAK cells may have some recognition mechanisms in common.

TNF-alpha can potentiate the effect of IFN on NK resistance, which correlates with the effect on MHC class I (MHC-I) expression of the target. Thus, the increased expression of MHC-I proteins is suggested to be an important factor of the IFN induced target resistance to NK activity.

The protective effect of MHC-I proteins may require accessory molecules. Studies were performed to characterize proteins associated with MHC-I. A major 90 kD protein and proteins of about 170 kD and 220 kD were found associated with MHC-I. These molecules are called 'MAMs', for MHC associated molecule. MHC class II (MHC-II) proteins were also associated with MHC-I, as well as with the MAMs. Thus, a model is proposed were MHC class I and II proteins form a big multimer complex together with the gp90, gp170 and gp220 MAMs on the cell surface.

Possible functions for the MAMs are discussed. They may represent target molecules for NK cells in the absence of MHC-I expression. They may prove to be important for signal transduction through MHC proteins, which probably requires accessory molecules for this function.

Results are presented, indicating that MHC-I molecules on the NK cells can provide negative signals that inhibit the NK cytotoxic activity. Signaling through MHC-I molecules could be an important regulatory factor for the interaction between NK cells and their targets.

Thus, the MHC-I expression on both NK cells and target cells are probably important for the NK cell-mediated cytotoxicity reaction.

June 1992

Monoclonal antibodies against plap and cytokeratin in radioimmunoscintigraphy and radioimmunotherapy—An experimental and clinical study

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Placental alkaline phosphatase (PLAP), is an oncodevelopmental antigen normally expressed in human placenta from the 9th week of gestation. PLAP is only expressed in trace amounts in the healthy adult, but the synthesis is increased 10- to 100-fold in more than half of ovarian carcinomas and in all seminomas.

A nude mouse model with HeLa cells xenografts was used to evaluate the value of using PLAP as a tumour marker in radioimmunoscintigraphy (RIS) and radioimmunotherapy (RIT). Three monoclonal antibodies (mab) against PLAP, and their Fab and $F(ab')_2$ fragments were evaluated. The usefulness of the mab H7 was demonstrated in both RIS and RIT. The accumulation of the ^{125}I-labelled H7 to the tumours was visualized by RIS, and the biological half-life in the tumours was estimated at 160 h.

Experimental RIT in the same nude mouse model, using H7 and an anticytokeratin mab (TS1), showed that both radiolabelled H7 and TS1 have properties that retard tumour growth. Autoradiographic studies showed that ^{125}I-labelled H7, and TS1 localized to different parts in the tumours. The tumour growth was retarded by both ^{131}I and ^{125}I-labelled H7 as well as TS1.

PLAP as well as other tumour antigens might be shed into the circulation where they can be quantified. A clinical study in patients with ovarian carcinomas was performed in order to evaluate PLAP compared to cancer antigen 125 (CA 125), as a serum marker for monitoring ovarian carcinomas. PLAP was less sensitive than CA 125 but could provide an additional information when both markers were used simultaneously.

This thesis indicates that the oncofoetal antigen PLAP and simple epithelial cytokeratins (CK 7, 8, 18, 19), can be useful targets for RIS and RIS, and that the mabs H7 and TS1 have properties which make them useful for both RIS and RIT.

June 1992

Benign and malignant parathyroid lesions in primary hyperparathyroidism

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Different types of primary hyperparathyroidism (PHPT) were analyzed with regard to morphologic features and biologic characteristics in benign and malignant parathyroid glands.

PHPT is a common endocrine disorder in the Western population. It predominantly affects postmenopausal women for unknown reasons. It is also the most common manifestation in the autosomal dominant syndrome multiple endocrine neoplasia type 1 (MEN1). Malignant transformation of the parathyroid glands is a rare phenomenon.

Parathyroid specimens (n-165), consisting of a) diseased glands from PHPT patients, b) normal gland biopsies from PHPT patients and c) normal gland biopsies from patients undergoing thyroid surgery, were analyzed for their content of estrogen, progesterone and glucocorticoid receptors. Ligand binding and immunological antibody techniques were used. Estrogen or progesterone receptor positivity (>0.05 fmol/μg DNA) overall was low (9%) in all types of glands examined. Glucocorticoid receptor positivity (>0.1 fmol/μg DNA) was found in 59% of all glands and predominantly in glands from patients with PHPT. There was also a significant difference in receptor content between normal and diseased glands.

In order to evaluate whether a common pathogenesis might exist for all types of MEN1 associated lesions, five MEN1 parathyroid, 24 sporadic PHPT and 27 pituitary tumors were analyzed for chromosome deletions, using restriction fragment length polymorphisms. Four of five familial and seven of 24 sporadic parathyroid tumors showed chromosome 11 losses compared to only two from the pituitary. Deletion mapping restricted the MEN1 gene locus telomeric to the PYGM locus at 11q13.

Different types of diseased parathyroid glands (MEN1 and sporadic) were analyzed regarding histopathology, DNA cytometry and allele losses. Single or multiple diseased glands did not differ with regard to morphology, DNA ploidy or allele losses. Glands from MEN1 patients did not differ from the sporadic PHPT ones. Both diploid and aneuploid tumors were found among the sporadic cases, whereas all familial tumors were diploid.

Ninety-five cases of parathyroid cancer were collected and reevaluated. Clinical, histopathologic and cytometric variables were assessed. The median follow-up time was 6 years. In 74 patients the diagnosis of carcinoma had been established at primary operation. Recurrences, as local or distant metastases, occurred in 40 patients. The median time to recurrence was 33 months (1–228) and 21 patients died within a median time of 2.5 years after the first relapse. The course of five patients with metastatic carcinoma was analyzed in detail. Invasive studies were most sensitive in localization. Hypocalcemic agents proved less effective compared to surgical intervention which often had sustained effect.

Histopathologic reevaluation confirmed the diagnosis of cancer in 41 cases whereas 15 additional cases were confirmed by the clinical course. The remaining 39 cases were classified as equivocal. Fibrosis, necrosis, nuclear atypia (especially macronucleoli), and mitotic figures were features more commonly encountered among the carcinomas. These variables also showed positive correlation with an aberrant DNA content demonstrated by image cytometry. Age and infiltrative growth were prognostic factors of importance for survival whereas the nuclear DNA pattern more closely related to time until recurrence.

June 1992

The pattern of metastases in human breast cancer—Methodological aspects and relation to prognostic factors

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Knowledge of factors which are responsible for the anatomical distribution of metastases in patients with breast cancer is largely unknown. The literature is reviewed on the subject of the author's research on both methodological aspects of detection of metastases and the relation of these metastases to the status of prognostic factors. The objects were to describe the anatomical pattern of metastases and to develop guide lines for optimal staging of patients with recurrent breast cancer.

Most patients develop recurrence within 2 years after the primary diagnosis. The most common sites of metastases are soft tissue (35%), bone (35%), lung (23%), pleura (12%), and liver (10%). The relative distribution of metastases in these sites is similar in different periods after mastectomy. The occurrence of local and regional recurrences is related to pathoanatomical features of the primary tumour and lymph nodes. The incidence of distant metastases is (negatively) correlated with tumour differentiation and steroid receptor content of the primary tumour. Other clinical and demographic characteristics, including the effect of systemic adjuvant therapy, are not, or only weakly, related to the subsequent pattern of metastases.

The evaluation of diagnostic methods should be carried out on well-defined and clinically relevant groups of patients. Many studies give no thorough information as to how the patients were selected. Although the sensitivity and specificity of the diagnostic tests are specified, it is not always possible to calculate the more clinically relevant diagnostic probabilities, i.e. the predictive values of a positive and a negative test. Rational staging of patients with recurrent breast cancer is achieved by screening the patients with physical examination, clinical chemical analyses, chest x-ray examination, and bone scintigraphy. If one of these tests is positive, the presence of metastases should be confirmed by other methods, such as x-ray examinations, bone biopsies and ultrasound or CT scans.

Most of the clinical research on breast cancer has dealt with identification of prognostic factors as well as the detection and elimination of metastases. Relatively few studies have focused on qualitative variations in the clinical course of disease, and even fewer studies consider factors related to the anatomical and temporal pattern of spread. Many studies have shown that prognostic factors detected at the time of primary diagnosis may also be related to the survival after recurrence. Future studies should be directed towards the effort to explain why or how these primary prognostic factors are related to the nature of metastatic disease, and therefore possibly also to the post-recurrent survival.

June 1992

Asbestos exposure and risk among asbestos cement workers—Studies of exposure and fibre concentrations, retention patterns and histopathological changes in lung tissue, and cancer risk, mortality and survival

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In a cohort of 3 144 asbestos cement workers the estimated median exposure was 1.2 fibres/ml air. In preserved lung tissue from necropsy, the 96 asbestos cement workers had higher asbestos fibre concentrations (transmission electron microscopy) than matched referents (medians: asbestos cement workers with mesothelioma 189, without mesothelioma 50, and referents 29×10^6 f/g dry weight). This was less pronounced for chrysotile than for amphibole (crocidolite, amosite, tremolite, anthophyllite) fibres, in spite of the fact that the exposure was mainly to chrysotile. Amphibole concentrations increased with duration of exposure. Asbestos bodies in lung tissue were associated both with duration of exposure and tissue concentrations of amphibole fibres. Further, associations were found between amphibole concentrations in lung tissue and fibrosis. Workers dead from mesothelioma had higher amphibole, but not higher chrysotile, concentrations than the others. Thus, the amphibole fibre concentrations and the number of asbestos bodies reflect risk.

The mortality from non-malignant (relative risk (RR) = 2.6), and malignant (RR = 2.5) respiratory disease was higher among the 1 929 male Swedish asbestos cement than in 1 233 referent workers. Dose–response relations with cumulated asbestos dose were found for pleural mesothelioma and colorectal cancer. The proportion of adenocarcinoma was high among asbestos cement workers with lung cancer and a high exposure (45%, referents 15%). Survival, as analysed by use of Cox's proportional hazards regression models, was a couple of years shorter among the asbestos cement than in the other industrial workers. A causal relation with the exposure is supported by the facts that the risk seemed to be confined to the period 20–40 years from start of employment, and was most pronounced among workers starting employment before the age of 30.

August 1992

On melphalan treatment in multiple myeloma—A clinical and experimental study

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Forty-nine patients with multiple myeloma, consecutively treated with orally administered melphalan and prednisone (induction therapy), were analysed retrospectively for treatment response. All patients obtained at least 6 courses of melphalan-prednisone. Treatment response, defined as a reduction of the myeloma protein with > 50%, was seen in 26 patients while 23 were non-responders. When treatment response was related to the dosage of melphalan given by mg/kg of body weight/week (dose-intensity), the numbers of responding and non-responding patients were similar in the group of patients receiving 'full dose' and in that with lower dose intensity. Drug-induced suppression of white blood cell, and platelet, count was similar in the responsing as well as in the non-responding group, indicating that the reason for non-response is not simply explained by deficient drug absorption. The cumulative dose of melphalan given during the induction therapy showed a positive correlation ($r = 0.47$, $p < 0.001$) to degree of response. Thus, the cumulative dose of melphalan given during induction therapy is of importance for the response, but cell bound factors regulating cell sensitivity also determine the individual responsiveness. Further studies of factors determining cytotoxicity and cell death warranted the use of a cell line.

Effects of melphalan on cell death, cell cycle progression, cell growth delay and DNA damage were studied in the human myeloma cell line RPMI 8226. The mechanism for the cytotoxic effect of melphalan was exerted in the S- and G_2-phases. Cell death occurred when the cells had left the G_1-phase and reached DNA replication. The growth delay effect was mediated by a transient 'G_2-block' related to formation and repair of interstrand cross-links. The latter effect dominates at lower dose intervals while the early effect with cell death was seen at higher doses of melphalan. We found that cell death after treatment with lower doses of melphalan could be obtained by the addition of corticosteroids (dexamethasone).

It is likely that time before initiation of DNA synthesis (G_1) and before mitosis (G_2) may regulate the sensitivity of tumour cells to melphalan treatment. Serum dependant growth delay was studied. In melphalan-treated serumfed cells we found cell cycle progression delay that may give time for repair of DNA damage. In serumstarved cells, a stimulation of outflow from G_1-phase, i.e. initiation of DNA synthesis, was found. As a result a long-standing 'G_2-block' occurred. In serumfed cells the 'G_2-block' is transient. It is likely that an extracellular factor, in this case a serumfactor, in some way mediates the inhibition of initiation of DNA-synthesis after melphalan induced DNA damage.

September 1992

An epidemiological approach to the etiology of Hodgkin's disease

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The aim of this thesis was to evaluate some factors of potential importance for the development and outcome of Hodgkin's disease (HD). A special interest has been focused on the cause of the immunodeficiency (ID) and its relation to HD.

The epidemiological features of HD in Cuba and Sweden were studied. HD in adult age was found to be more common in Sweden than in Cuba. The incidence of HD declined in Sweden after 1973 but remained stable in Cuba. The bimodal age-specific incidence pattern seen in industrialized countries was also observed in Sweden while in Cuba a shift from an unimodal to a bimodal pattern was recorded. This change was paralleled by the Cuban development from an agricultural to a more industrialized country. The data support the hypothesis of an intermediate incidence pattern in HD preceding that seen in highly developed countries.

In order to test the hypothesis of a causal relationship between blood donation and development of HD and other tumors, the incidence of cancer among 37 795 blood donors was studied. The total number of malignancies in this cohort was lower than that expected. No increased risk of HD was found. Blood donors may lead a more healthy life than the rest of the population explaining at least in part their lower cancer indicence.

Lymphocyte functions and T cell counts and subpopulations were related to clinical characteristics and prognosis in 262 HD patients. Patients with an increased spontaneous and decreased Concanavalin A induced blood lymphocyte DNA synthesis had a

poor prognosis. In multivariate analysis lymphocyte function was the strongest prognostic factor besides age.

To study the possible existence of genetic/environmental factors contributing to the ID associated with HD, 65 first-degree relatives and 12 spouses to 21 consecutive patients with HD were studied. Moreover, 7 twin pairs in which one partner had HD and 4 additional healthy co-twins to deceased HD patients were included. Blood lymphocyte abnormalities were found in 32% consanguineous and 17% non-consanguineous healthy relatives and in 50% healthy co-twins as compared to 16% healthy controls supporting the concept of genetic factors contributing to the ID in HD.

Epstein–Barr virus (EBV) serology was studied in 61 untreated HD patients and related to clinical characteristics. An elevated antibody titer to virus capsid antigen (VCA; IgG) was observed. Patients with advanced and relapsing disease had higher titers to VCA (IgG) and EBNA as compared to patients with limited disease and those who remained in complete remission. In addition, EBV serology was studied in 38 patients during the course of disease. Continuously elevated anti-VCA (IgG) titers were observed. Therapy (chemotherapy in particular) seemed to lower elevated pretreatment antibody levels to EBV only temporarily.

Patients with HD have an abnormal cell mediated immunity, which in part is related to a poor prognosis. A fraction of close relatives also showed such defects. Epidemiological and other data may suggest a causal relationship between EBV and HD. However, EBV and impaired immunological reactivity of HD patients may represent co-factors that enable environmental and/or genetic factors to act in the evolution and modulate the course of the disease.

September 1992